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Antiparasitic Drugs. II. Anticoccidial Activity of 4,5 imidazoledicarboxamide and Related Compounds. (23977)

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Coccidiosis is an important protozoan disease of poultry which "costs poultry raisers millions of dollars each year because of unthriftiness, poor utilization of feed, loss of weight, and death of infected birds"(1). During the past 2 decades several effective chemotherapeutic agents have been discovered for prevention and control of coccidiosis in poultry and other domestic animals. Nevertheless, there has been a constant search for more effective and better tolerated drugs for use as coccidiostats. We recently found that 4, 5-imidazoledicarboxamide(2) (Fig. 1) is a potent and well-tolerated coccidiostat. The compound has been assigned the generic name of glycarbylamide.* This paper reports the results of chemical and biological studies on glycarbylamide and related compounds.

Materials and methods. Chemical. 4,5-Bis (N-acetylcarbamyl)-imidazole(V). Two g of 4,5-imidazoledicarboxamide was refluxed for 15 hours in a mixture of 100 ml of acetic anhydride and 10 ml of acetic acid. The reaction mixture was then concentrated to dryness *in vacuo* at 100°. The residue thus obtained was slurried with water, the slurry allowed to stand at room temperature for about an hour and then filtered. Two g of product was obtained. After recrystallization from methanol it melted at 259-60°. *Anal.* Calcd. for $C_9H_{10}O_4N_4$: C, 45.38; H, 4.23; N, 23.53; N.E. 238. Found: C, 45.42; H, 4.26; N, 23.77; N.E. 241. *4,5-Bis (N-propionylcarbamyl)-imidazole (VI).* This compound, prepared by the same general method as V, melted at 239-40° after recrystallization from ethanol. *Anal.* Calcd. for $C_{11}H_{14}O_4N_4$: C, 49.61; H, 5.30. Found: C, 49.36; H, 5.27. *4-Acetylcarbamyl-5-imidazolecarboxamide (IV).* Two and four-tenths g of 4,5-bis (N-acetylcarbamyl)-imidazole was suspended in 25 ml of water and 10 ml of N sodium hydroxide was added with vigorous shaking. The clear solution obtained stood at room temperature for about 60 hours during which time the product slowly precipitated. When

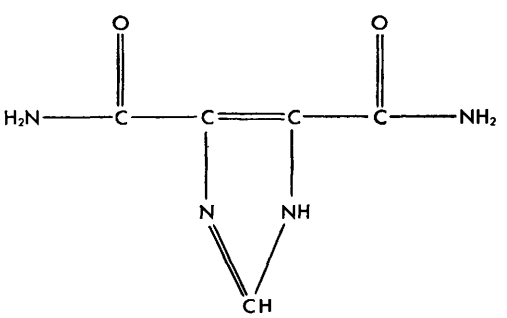


FIG. 1. 4,5-Imidazoledicarboxamide (Glycarbylamide).

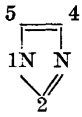
* 'GLYCAMIDE' is the trademark of Merck & Co., for this compound.

filtered and recrystallized from methanol, it melted at 285°. *Anal.* Calcd. for $C_7H_8O_3N_4$: N, 28.56. Found: N, 29.19. *N^4,N^5-Dimethyl-4,5-imidazoledicarboxamide (IX).* This was prepared from 4,5-imidazoledicarboxylic acid dimethyl ester and 40% aqueous methylamine; m.p. 223°. *Anal.* Calcd. for $C_7H_{10}O_2N_4$: C, 46.14; H, 5.53. Found: C, 46.38; H, 5.33. *4,5-Imidazoledicarbonitrile (XII).* A mixture of 10 g of 4,5-imidazoledicarboxamide and 60 ml of phosphorus oxychloride was heated for 2 hours on a steam bath. Excess phosphorus oxychloride was then removed *in vacuo*. Sufficient ice water was added to the residue to permit mixing. Sodium bicarbonate was then added until only a weak acid reaction was observed. The cold mixture was filtered. The precipitate gave 7 g of product on extraction with hot ether. Extraction of filtrate with ether yielded an additional 1.5 g. The combined extractives, recrystallized from 25 ml of boiling water, gave 5.3 g of product, softening at 173°, melting at 176-177°. Woodward(3) reports a melting point of 175°. *1-Ethyl-4,5-imidazoledicarboxamide (XIV).* To a solution prepared by dissolving 3.6 g of sodium metal in 900 ml of methanol were added successively 27.3 g of methyl-4,5-imidazoledicarboxylate and 25 g of ethyl iodide. The mixture was refluxed for 16 hours, then concentrated *in vacuo* to a syrupy liquid. The liquid was treated with 300 ml of concentrated ammonium hydroxide, mixed and let stand 60 hours. After this time the precipitated solid was filtered off, washed with a little cold water and air dried. The yield of crude product was 17 g, m.p. 215°. The material was dissolved in 850 ml of boiling absolute alcohol, charcoaled and filtered. After concentration to about two-thirds volume, the solution when cooled in an ice bath precipitated 12.1 g of 1-ethyl-4,5-imidazoledicarboxamide, m.p. 215-6°. *Anal.* Calcd. for $C_7H_{10}O_2N_4$: N, 30.75. Found: N, 30.89. *1-Benzyl-4,5-imidazoledicarboxamide (XV).* To a solution prepared by dissolving 6 g of potassium metal in 250 ml of methanol were added successively 25 g of methyl-4,5-imidazoledicarboxylate and 26 g of benzyl bromide. The mixture was refluxed with stirring for 9 hours. Precipitated potassium bromide was recovered by filtration. The filtrate was concentrated *in vacuo* to a syrupy liquid and to this was added 300 ml of concentrated ammonium hydroxide. After standing 48 hours, precipitated solid was filtered off. The air-dried product was dissolved in 1.8 L of hot acetone, a small amount of insoluble material being rejected. The acetone solution was concentrated and filtered. Crystallization gave 21.6 g of product melting at 203-5°. On recrystallization from methanol the 1-benzyl-4,5-imidazoledicarboxamide melted at 204-6°. *Anal.* Calcd. for $C_{12}H_{12}O_2N_4$: N, 22.94. Found: N, 23.14.

Biological. Experimental cecal coccidiosis was produced in 2-week-old chicks as follows: Straight run White Leghorn chicks, in groups of 3 each, were weighed and placed in cages with wire floors. They were fed *ad lib.*, a standard laboratory ration in which graded concentrations of test-chemicals were carefully blended just prior to use. Nicarbazin (4) was used as the reference standard. The normal and infected control birds were fed basal ration. On the second day of test, the chicks were each orally inoculated with 50,000 sporulated oocysts of *Eimeria tenella*. Papers under cages were examined on 6th, 7th and 8th days of assay for presence or absence of bloody droppings. A score of 0 was given if no bloody spots were observed; scores of 1, 2 or 4 were assigned for 1-3, 4-6 and >6 bloody spots, respectively. On 8th day of assay, the surviving birds were weighed, sacrificed and examined grossly for cecal coccidiosis lesions. To eliminate bias, the identity of groups under examination was not known to the observer. Normal ceca were scored 0 and ceca with detectable, moderate or maximal lesions were scored, 1, 2 and 4, respectively. When a bird died, and cecal coccidiosis lesions were present, a score of 5 was recorded. If the total of the 2 scores was 0-5, a rating of "active" was given to the compound; if the total score was 6 or more the compound was rated "inactive," at the concentration tested.

Results. The relative anticoccidial potency of 17 imidazole compounds is shown in Table I. None was as potent as 4,5-imidazoledicar-

TABLE I. Anticoccidial Potency of 4,5-Imidazoledicarboxamide and Related Compounds.



Compound	Substituents on imidazole ring 1	2	4	5	% in feed	Score rating, active & inactive	% relative potency (compared to 4,5-imidazoledicarboxamide)	References
I	H	H	H	H	.2	I‡	< 1	†
II	H	H	$CONH_2$	H	.2	A	1	5
III	H	H	"	$CONH_2$	.002	A	100	2
IV	H	H	$CONHCOCH_3$	"	.008	A	25	*
V	H	H	"	$CONHCOCH_3$	.004	A	50	*
VI	H	H	$CONHCOC_2H_5$	$CONHCOC_2H_5$	.004	A	50	*
VII	H	H	COOH	COOH	.2	I	< 1	†
VIII	H	H	$COOCH_3$	$COOCH_3$	.2	I	< 1	2
IX	H	H	$CONHCH_3$	$CONHCH_3$	.05	I	< 4	*
X	H	H	$CONHNH_2$	$CONHNH_2$	.02	I	<10	6
XI	H	H	CONHNHCO		.02	I	<10	6
XII	H	H	CN	CN	.04	I	< 5	3, *
XIII	CH_3	H	$CONH_2$	$CONH_2$	.02	A	10	2
XIV	C_2H_5	H	"	"	.005	A	40	*
XV	$C_6H_5CH_2$	H	"	"	.02	A	10	*
XVI	β-D-ribofuranosyl	H	"	"	.01	I	<20	7
XVII	H	CH_3	"	"	.04	I	< 5	8

* Described in this paper. † Commercial sample, Eastman Kodak Co. ‡ A = active, I = inactive.

boxamide (III), which was active at 0.002% concentration in the feed. Imidazole itself had no activity; imidazole-4-carboxamide (II) was much less potent than the dicarboxamide. In compounds IV, V and VI, derivatives of 4, 5-imidazoledicarboxamide in which one or both amide groups were acylated, activity was retained, but other 4,5-substituted imidazoles closely related to the dicarboxamide, 4,5-imidazoledicarboxylic acid and its dimethyl ester (VII, VIII), the bis (N-methyl-carboxamide) (IX), the dihydrazide (X) and cyclic hydrazide (4.7-dihydroxy-1,3,5,6-tetraazaindene, XI) and the dinitrile (XII), were all completely inactive. Replacement of hydrogen at position 2 of 4,5-imidazoledicarboxamide by methyl, as in XVII, likewise resulted in loss of anticoccidial effectiveness. The 1-methyl and 1-ethyl derivatives of 4,5-imidazoledicarboxamide (XIII, XIV) were effective coccidiostats but less potent than glycarbylamide (III). Replacement of 1-methyl with 1-benzyl gave a diamide (XV) of equal effectiveness.

Glycarbylamide was approximately 4 times more potent than nicarbazin for *E. tenella* and was highly effective for coccidial infections produced by *Eimeria acervulina* and *Eimeria necatrix* (not yet published.)

The possibility of interference with purine biosynthesis was our first consideration in connection with the mechanism of action of 4,5-imidazoledicarboxamide. Aside from the labile ribose phosphate unit, the dicarboxamide differs from 5-formamido-4-imidazolecarboxamide ribotide(9,10) only in the substitution of 5-$CONH_2$ for 5-NHCHO. However simultaneous administration of either 5-amino or 5-formamido-4-imidazolecarboxamide with 4,5-imidazoledicarboxamide had no effect upon the anticoccidial action of the latter compound. 1-β-D-Ribofuranosyl-4,5-imidazoledicarboxamide (XVI) was inactive at 0.01%. The possibility that the anticoccidial action of 4,5-imidazoledicarboxamide was due to a purine antagonist function was further tested by simultaneous administration of xanthine, hypoxanthine, guanine, adenine and uric acid and the coccidiostat. No effect on therapeutic potency was observed with any of these combinations.

Summary. 1. Preparation of 7 substituted

imidazoles has been described. 2. Anticoccidial activity was demonstrated for imidazole-4-carboxamide and certain related compounds. 3. The most potent compound was 4,5-imidazoledicarboxamide (glycarbylamide). 4. Replacement of one or both carboxamides in the 4,5 positions on the imidazole ring reduced or completely eliminated the anticoccidial activity of glycarbylamide. Substitutions at the 1 or 2 positions on the imidazole ring produced compounds of less activity than glycarbylamide.

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Further Properties of Isoglutamine-oxytocin; Inhibition of Pressor Activity of Vasopressin.* (23978)

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The synthesis of the isoglutamine isomer of oxytocin, the chief oxytocic principle of the posterior pituitary gland, has recently been described(1). This isomer, the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-isoglutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucyl-glycinamide, differs from oxytocin by substitution of an isoglutamine residue for the glutamine residue in the hormone. In view of this difference in structure and our interest in the correlation of the structures and properties of such polypeptides, the isomeric compound was compared with oxytocin. Observations were made on distribution coefficients, optical rotation, electrophoretic mobility, molecular weight and infrared pattern(1). In addition, the isomer was tested for some physiological activities associated with oxytocin; namely, oxytocic activity, avian vasodepressor activity, and pressor activity in the rat. As has been already reported, these activities were not detected in the isomer. Further testing revealed that isoglutamine-oxytocin exerts a definite inhibitory effect on the pressor action of administered vasopressin in the anesthetized rat. Inhibition toward both purified arginine-vasopressin and the posterior pituitary standard powder was observed. It may be recalled that oxytocin can be converted into a crystalline flavianate derivative (2). Efforts were therefore made to determine whether the isoglutamine isomer could show similar behavior. It has now been found that isoglutamine-oxytocin also yields a crystalline flavianate, and that the latter differs in form from the corresponding oxytocin derivative. When the acetate of isoglutamine-oxytocin was rigorously dried, it underwent change, as indicated by decrease in solubility. Results of elementary analysis and molecular weight determination suggested that it had probably lost acetic acid.

Experimental. Inhibition of pressor action of vasopressin. Anesthetized rats weighing 290-480 g were injected intravenously with solutions of isoglutamine-oxytocin(1), vasopressin, and various mixtures of these. The

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